Novel N-Terminal Variant of Human VDR

Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, New South Wales, 2010 Australia

The importance of N-terminal regions of nuclear hormone receptors in transcriptional regulation is increasingly recognized. As variant VDR gene transcripts indicated possible N-terminally extended receptors, we investigated their natural occurrence, transactivation capacity, and subcellular localization. A novel 54-kDa VDRB1 protein, in addition to the previously recognized 48-kDa VDRA form, was detected in human kidney tissue as well as in osteoblastic (MG63), intestinal (Int-407, DLD-1, and COLO 205F), and kidney epithelial (786) human cell lines by Western blots using isoform-specific and nonselective anti-VDR antibodies. VDRB1 was present at approximately one-third the level of VDRA. Isoform-specific VDRB1 expression constructs produced lower ligand-dependent transactivation capacity, and subcellular localization patterns of the green fluorescent protein-tagged VDR isoforms differed. VDRB1 appeared as discrete intranuclear foci in the absence of 1,25-dihydroxyvitamin D$_3$, whereas VDRA produced diffuse nuclear fluorescence. After 1,25-dihydroxyvitamin D$_3$ treatment, both VDR isoforms exhibited similar diffuse nuclear signal. In the absence of 1,25-dihydroxyvitamin D$_3$, the VDRB1 foci partially colocalized with SC-35 speckles and a subset of promyelocytic leukemia nuclear bodies. These data provide the first evidence of VDRB1, a novel N-terminally variant human VDR that is expressed at a level comparable to VDRA in human tissue and cell lines. It is characterized by reduced transactivation activity and a ligand-responsive speckled intranuclear localization. The intranuclear compartmentalization and altered functional activity of VDRB1 may mediate a specialized physiological role for this receptor isoform. (Molecular Endocrinology 15: 1599–1609, 2001)

THE BIOLOGICALLY ACTIVE form of vitamin D, 1,25–1,25-dihydroxyvitamin D$_3$ [1,25-(OH)$_2$D$_3$], acts through the VDR, a member of the nuclear hormone receptor (NHR) superfamily, which includes steroid, thyroid, retinoid, and orphan receptors. Upon ligand binding, VDR forms homodimers or VDR:RXR heterodimers that bind to specific vitamin D-responsive elements, recruit additional coactivators, and interact with the general transcription apparatus to initiate or inhibit gene transcription (1). Vitamin D regulates calcium homeostasis as well as specialized functions in cell proliferation and differentiation (2). Multiple species of ERs, TRs, RARs, and RXRs have been reported to be derived from separate genes. By contrast, variant PRRs and PPARs have been reported to be generated through differential promoter usage and/or alternative splicing (3–8). The functional diversity contributed by these variants is increasingly understood. For example, the human PR isoforms exert striking differences in promoter specificity, suggesting that the two forms have a different potential to synergize with one another and/or other factors involved in modulation of transcription (4). The PR isoforms are also unique in that only B receptors can activate transcription in the presence of antiestrogens (9, 10); furthermore, A receptors can dominantly inhibit B receptors (8, 11) as well as other members of the steroid receptor superfamily (12, 13). Overall, the existence of these isoforms suggests that variant receptors may modulate different physiological responses.

These alternate receptors vary in the length of their N-terminal A/B domains, the region of greatest diversity in the NHR (1), from 23 to more than 600 amino acids (aa) (14, 15). Although vitamin D exhibits functional diversity, the VDR differs from the other NHRs with its very short A/B domain (23 aa) and limited structural variability (1, 2, 14). Two VDR isoforms differing in the N terminus by 14 aa and generated by alternative translation initiation have been described in avian species (16). Until recently, there has been limited evidence for human VDR (hVDR) isoforms. A 3-aa N-terminal extension due to a common start codon polymorphism (17) has been reported to cause elevated transactivation activity in some studies (18). A dominant-negative VDR generated by intron 8 retention in the rat (19) has uncertain physiological significance. Overall, however, there has been little evidence of functionally different isoforms of the hVDR.

Recently, we reported alternatively spliced upstream exons in the hVDR gene locus with the potential to encode variant proteins termed VDRB1 and
VDRB2, with 50- or 23-aa N-terminal extensions, respectively (14). There was one variant transcript potentially encoding each variant protein isoform, compared with six transcripts encoding the standard A form of VDR in all tissues and cell lines examined, as well as four additional VDRA transcripts initiating at a distal promoter that was active in the major vitamin D target tissues (14). In the present study we report that the novel VDRB1 protein coexists with the previously identified VDRA, and show that it has altered ability to transactivate a vitamin D-responsive promoter-reporter construct. Based on studies with chimeric green fluorescent protein (GFP)-VDR proteins, we also provide evidence that VDRB1 localizes to unique intranuclear foci, unlike the uniform distribution of VDRA. The ligand response of the VDRB1 foci differs from those reported for other NHRs (20–24). The VDRB1 foci associate with a subset of SC-35 splicing factor domains, but not with PML or p80 coilin.

**RESULTS**

**Immunodetection of hVDR Isoforms**

Initial Western blots did not show the presence of additional band(s) in the predicted size range. Thus, large quantities of whole-cell lysate were analyzed. Using this approach, two immunoreactive proteins corresponding in size to transfected VDRA and VDRB1 were detected in empty vector transfected MG63 whole-cell lysates by the commercial anti-VDR antibody 9A7 (Fig. 2A). These identities were confirmed using isoform-specific antisera with reactivities previously demonstrated by probing Western blots of extracts from COS-1 cells expressing individual VDR isoforms (Fig. 1). Both the VDRB-common and the VDRB1-specific antisera detected only the upper band in the MG63 lysate but bound appropriately to VDRB1 and VDRB2 in transfected cell lysates (Fig. 2B and C). VDRA and VDRB1 were also present in the intestinal cell lines Int-407, COLO 206F, and DLD-1, as well as the 786 kidney cell line (Fig. 3A). Furthermore, both VDRA and VDRB1 were detected in human kidney (Fig. 3B). Quantitation of band intensities suggested that VDRB1 is present at approximately one-third the level of VDRA in MG63 cells, with comparable intensities for both isoforms in kidney tissue and the other cell lines. A band of the predicted size and immunoreactivity of the VDRB2 protein was not detected in any sample.

**VDR Isoform Transactivation**

Hormone-stimulated transactivation by the novel receptor VDRB1 was lower than that of VDRA on the rat 24-hydroxylase reporter construct in two cell lines with low endogenous VDR. In COS-1 monkey kidney cells, hormone-stimulated transactivation by the N-terminally extended VDRB1 was 60% of the level of VDRA (Fig. 4A), despite comparable protein expression levels (Fig. 4C). Transactivation was similarly less efficient (~60% of the VDRA level) in the P19 mouse embryonal carcinoma cell line (Fig. 4B). There was no consistent effect of either VDR isoform on basal transcriptional levels in either cell line, indicating that there was neither ligand-independent transcriptional activation nor repression by either isoform.

**Ligand-Regulated Subcellular Localization of hVDR-Enhanced Green Fluorescent Protein (EGFP) Fusion Proteins**

Short stretches of basic amino acids (RNKKR and RPHRR) in the N terminus of VDRB1 resemble a typical nuclear localization consensus sequence (14, 25–27); therefore, intracellular localization was examined by transient expression of VDR-EGFP fusion proteins in COS-1 cells. Protein bands of appropriate sizes (75 and 81 kDa for VDRA- and VDRB1-EGFP fusion proteins, respectively) were detected by both anti-GFP and 9A7 anti-VDR antibodies (Fig. 5, A and B). These fusion proteins were transcriptionally active, with the VDRB1 exhibiting less 1,25-(OH)2D3-stimulated transactivation than the VDRA fusion protein, as seen with the unmodified VDR isoforms (Fig. 5C).

VDRB1-EGFP appeared as distinct nuclear foci with little signal in the cytoplasm (Fig. 6, A and C). All VDRB1-EGFP expressing cells with a strong signal exhibited the distinct punctate distribution. A small proportion of cells (15%) exhibited a homogeneous faint signal comparable to EGFP alone. Bright homogeneous nuclear signal was seen in all VDRA-EGFP expressing cells (Fig. 6, B and D). Both proteins were excluded from nucleoli. Varying VDR plasmid concentration (0.02–2 μg) or altering time before fixation (24–72 h posttransfection) had no effect on the punctate distribution of either VDR isoform. After exposure to 1,25-(OH)2D3 (18 h), the VDRB1-EGFP nuclear pattern had become diffuse with more cytoplasmic signal apparent. Nuclear VDRA-EGFP signal was not significantly altered by ligand treatment, although the level and distribution of cytoplasmic signal were more variable (Fig. 6, B and D, lower panel).

**Immunofluorescence of VDR Isoforms with Nuclear Speckled Proteins, Sites of Transcription, and RNA Processing**

The VDRB1-GFP foci were compared with the speckles of active RNA Polymerase II (RNA PolII) (28–31), SC-35, the non-small ribonucleoprotein spliceosome assembly factor (32, 33), p80 coilin, which localizes to sites of RNA processing (31, 34–36) and PODs (PML oncogenic domains), which are discrete interchromosomal accumulations of several proteins including PML and Sp100 (37–39). The localization patterns of...
VDR and TATA Binding Protein (TBP) and RNA PolIIo were examined using the GFP-tagged VDR isoforms and immunostaining for endogenous TBP and RNA PolIIo. There was incomplete nuclear overlap between TBP and both VDR isoforms, as indicated by the non-uniform staining in the merged images (Fig. 6, A and B). Although treatment with 1,25-(OH)₂D₃ dispersed the characteristic VDRB1 speckles, thereby increasing the extent of overlap with TBP, the general background of mottled TBP nuclear staining was not altered with 1,25-(OH)₂D₃ treatment. Hence, the degree of colocalization was due merely to the generally homogenous nuclear distribution of both proteins rather than specific association. Coincidental overlap with either VDR isoform and RNA PolIIo was also due to the even distribution of active RNA PolIIo throughout the nucleoplasm, irrespective of 1,25-(OH)₂D₃ status (Fig. 6, C and D). The degree of colocalization was increased upon 1,25-(OH)₂D₃ treatment, with both VDR isoforms exhibiting a uniform distribution overlying that of active RNA PolIIo, although the enhancement of apparent overlap upon treatment was more striking for VDRB1.
There was a clear correlation between the GFP-tagged VDRB1 and SC-35 indirect immunofluorescence signals (Fig. 7A). Each of the VDRB1 foci was immediately adjacent to but not absolutely colocalized with an SC-35 speckle, but not every SC-35 speckle was associated with a VDRB1 focus. In contrast, VDRB1 and PML nuclear body signals were only coincidentally colocalized, as the VDRB1 foci only partially associated with the PML domains; their distribution was randomized in comparison to the PODs (Fig. 7B). There was no detectable colocalization between VDRB1 and the punctate pattern of protein p80 coilin (Fig. 7C). Detection of physical associations is precluded by limitations of the resolution of indirect immunofluorescence and is beyond the scope of this study. Apparent overlap of VDRA and SC-35 speckles, PML domains, or coiled bodies was due to the uniform distribution of the VDR and coincidental (data not shown).

DISCUSSION

This study provides evidence for a previously unknown variant of the hVDR that corresponds in size and immunoreactivity to the predicted 54-kDa VDRB1 protein (14). VDRB1 was present at approximately a 1:3 ratio with the well studied 48-kDa VDRA protein in human kidney and in the five cell lines examined. These findings suggest a role for posttranscriptional regulation in determining variant VDR protein levels, as the number of VDRA-encoding transcripts (ten) exceeds the single transcript encoding VDRB1. A role for posttranscriptional regulation of VDR isoform expression is further supported by the apparent absence of the predicted VDRB2 isoform, despite the previously reported evidence for VDRB2-encoding transcripts (14). It is possible that VDRB2 may exist at a level lower than was detected by the current analyses. Our preliminary studies indicate that this receptor would produce a level of transactivation intermediate between VDRA and VDRB1 (our unpublished observations).

The N-terminal domains of other NHRs modify transcriptional activity by interacting with basal transcription machinery (40–42), serving as phosphorylation substrates (43, 44), or functioning as activation function-1 domains (45–47). In agreement with this pattern, transactivation by VDRB1 was clearly lower than by VDRA on the rat 24-hydroxylase promoter (T.-A. Cock, manuscript submitted). This difference was seen in both COS-1 and P19 cells, indicating that the effect of the N-terminal extension on transactivation function is not cell line specific.

The VDRB1-EGFP fusion protein accumulated in nuclear speckles that were not seen with VDRA-EGFP. Ligand treatment dispersed VDRB1 to a homogenous distribution similar to that of VDRA-EGFP. The ligand sensitivity of the speckles suggests functional significance, as proposed for other NHRs that are also distributed in dynamic clusters or foci (20, 48). Importantly, however, the VDRB1 speckle distribution and ligand response pattern differed from that reported for other receptors such as GR and MR, which accumulate in discrete nuclear clusters only after ligand treatment (20, 48–52).

The distribution of the VDRA-EGFP fusion protein throughout the nucleus and cytoplasm in the absence of ligand is similar to that of an analogous fusion protein reported elsewhere (53). In that study, fluorescence became entirely nuclear and homogeneous within 30 min of 1,25-(OH)2D3 treatment, and all cytoplasmic signal was restored within 30 min of hormone removal. These findings are consistent with cell fractionation and immunocytology studies, which indicated that VDR was both nuclear and cytoplasmic (20, 48, 49, 54). More recently, standard VDR (i.e. VDRA) has been shown to form ligand-induced intranuclear foci upon heterodimerization with RXR, consistent with a role for heterodimer binding to DNA target sites in the formation of these foci (55). The foci seen upon heterodimerization with RXR, however, do not resemble the VDRB1 foci, and endogenous RXR did not colocalize with GFP-VDRB1 in the present study (data not shown).

NHR nuclear localization and distribution in discrete nuclear domains appear to be important aspects of...
functional coordination (56). TRβ1 is present in both the nucleus and the cytoplasm in the absence of ligand but translocates to the nucleus and is uniformly distributed in the presence of T3 (57). In contrast, MR is predominantly nuclear in the absence of ligand but concentrates to prominent clusters within the nucleus when bound to aldosterone (22). Similarly, unliganded GR resides predominantly in the cytoplasm, with hormone activation leading to translocation to the nucleus and gene activation (50). Transcriptional activation of GR by ligand is also correlated with focal nuclear accumulation similar to MR (20). In colocalization studies of VDRB1 and GR, we have also seen nuclear clustering of GR within 15 min of dexamethasone treatment, but this punctate distribution did not colocalize with the VDRB1 speckles, which themselves were not affected by dexamethasone treatment (data not shown).

Nuclear accumulation may be attributable to chromatin or hormone response element targeting (23), but more complex intranuclear interactions are also possible (52, 58). Proficient transcription and RNA processing may occur at the periphery of speckles (29, 32, 59, 60) or nuclear foci may be storage sites of splicing and transcription factors distal from the sites of bulk transcription (61). The VDRB1 speckles could therefore relate to regulation of specific genes, as has been suggested for clustered GR molecules, or may represent sites of receptor storage.

Mammalian nuclei contain subnuclear foci of various transcription factors, hnRNP proteins, heterochromatin proteins, and even elements of the cleavage and

---

**Fig. 3.** Detection of 54-kDa VDRB1 in Human Cell Lines and Tissue

Whole-cell lysates (400 μg protein) from Int-407, COLO 206F, DLD-1, and 786 cells were run on a 12% SDS-PAGE, transferred to PVDF membrane, and probed with the anti-VDR antibody 9A7 (A), or VDRB1-specific antiserum (B). Whole-cell protein lysates from human kidney (300 μg protein) were compared with individual VDR isoform-transfected protein lysates (7 μg protein) and probed with the anti-VDR antibody 9A7 (C) VDRB1-specific antiserum (D).
polyadenylation machinery (38). The various morphologically distinct substructures, or nuclear bodies, include sphere organelles, interchromatin granule clusters, coiled bodies, and the PML nuclear bodies or PODs (61–63). The emerging view is that many of these subdomains are associated with specific genetic loci and that interactions with these various domains and loci are dynamic and can change in response to cellular signals (38).

A number of nuclear proteins have been associated with a speckled localization, including SC-35, PML, ND10, and Sp100 (37, 38, 64). The VDRB1 foci were associated with the periphery of the SC-35 protein and that 1,25-(OH)2D3-induced release of VDRB1 from speckles may modulate the cellular response to ligand. Differential transcriptional activity of the VDR isoforms could preferentially modulate subsets of target genes in vitamin D-responsive pathways.

**MATERIALS AND METHODS**

Production and Characterization of Isoform-Specific Antipeptide Antibodies

Polyclonal B-common and B1-specific antisera were generated by intraperitoneal injection of rabbits with 300 μg of KLH-coupled synthetic peptides in Freunds complete adjuvant. The peptides were NH2-MEWRNKKRSDWLSMVR TAGC-COOH encoded by exon 1d hVDR, NH2-SVRPHRAPLGSTYLPPAPSC-COOH encoded by exon 1c (Fig. 1A). Animals were boosted and bled at 4-wk intervals with a terminal bleed after 7 months (Chiron Corp. Technologies, Melbourne, Australia). Antibody titers were determined by ELISA and specificities confirmed by Western blots of lysates prepared from COS-1 cells transfected with individual isoform-specific expression constructs (see below). Lysate protein (5 μg) was electrophoresed on a 10% SDS-PAGE and immunoblotted on polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membrane was sequentially probed with the test antisera. The 9A7 antibody detected all VDR forms, whereas the B-common antiserum detected both B form VDRs but not VDRA; and the B1-specific antiserum detected only VDRB1 (Fig. 1C).

**Isoform-Specific Constructs**

hVDR A, B1 and B2 cDNAs were cloned from SAOS-2 osteosarcoma RNA by RT-PCR. Forward primer for VDRA was 5'-GAGTCAGCTTTCAAGGGATGAGGAGGAGAATTGC-3' and for VDRB1 and VDRB2 was 5'-GAGTCAGCTTTCAAGGGATGAGGAGGAGAATTGC-3'. The common reverse primer from exon 9 was 5'-GACTCGGCGCCTTCTAGTCGAGATCTCATT-3'.
To ensure translation of only a single variant from a specific ATG codon in each expression construct, noninitiating methionine codons in exons 1d and 1c were mutated to isoleucine, using forward PCR primers (M23I 5'-TGGCTGTC-GATTGTGCTCAGAAC-3' and M60I 5'-TCAGGGATTGAG-GCAATTGCGGCC-3') and the common reverse primer.

For expression studies, modified cDNAs were cloned into pRC-CMV (Stratagene, La Jolla, CA). For subcellular localization, the cDNAs were subcloned into pEGFP-N1 vector (CLONTECH Laboratories, Inc., Palo Alto, CA). All plasmids were verified by DNA sequencing (Perkin-Elmer Corp., Norwalk, CT).

Cell Extract Preparation and Immunoblotting

Human osteosarcoma MG63, embryonal intestinal Int-407, colon carcinoma DLD-1 and COLO 206F, and renal adenocarcinoma 786 cell lines, all known to express transcripts encoding the putative VDR isoforms (14), were grown in DMEM with 10% FBS. For isoform size standards, MG63 cells were transfected in 15-cm dishes with 5 μg pRC-CMV-VDR isoform constructs using FUGENE6 reagent (Roche Molecular Biochemicals, Mannheim GmbH, Germany). Whole-cell extracts were prepared from untransfected cultures, and 48 h after transfection, cells were harvested, washed in 1×PBS, and resuspended in boiling SDS-lysis buffer (10% SDS, 0.15 M Tris-HCl, 1% β-mercaptoethanol) with 10% DNase/RNase, and vortexed for 30 sec (72). The lysates were then boiled for 30 sec and immersed in liquid nitrogen. Samples were lyophilized on a Speedvac drier and resuspended in rehydration buffer [8 M urea, 2% dithiothreitol (DTT), 2% 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propane sulfonate (CHAPS)]. Total protein was separated by 12% SDS-PAGE, transferred to PVDF membrane, and blocked overnight at 4°C in Tris-buffered saline with Triton-X100 containing 10% skim milk powder, 0.5% BSA before incubation in isofrom-specific antiserum (1 μg/ml) or with 0.4 μg/ml 9A7 rat anti-VDR monoclonal (Affinity BioReagents, Inc., Golden, CO) as primary antibody for 2 h at room temperature, followed by incubation with donkey antirabbit horseradish peroxidase (HRP) or goat antirat-HRP secondary antibody (Zymed Laboratories, Inc., South San Francisco, CA) for 1.5 h at room temperature. Immunoactive proteins were identified by enhanced chemiluminescence (Amersham Pharmacia Biotech). Band intensities were estimated by densitometry of a lightly exposed film containing a serial dilution (400–10 μg protein) of MG63 cell lysate (Molecular Dynamics, Inc., Sunnyvale, CA; Amersham Pharmacia Biotech).

Total Protein Preparation from Human Tissue

Frozen tissue was ground to a fine powder using a ceramic mortar and pestle kept cool under liquid nitrogen immersion. Approximately 500 μl of dry powder were transferred
to a clean Eppendorf tube and washed three times in low-salt washing buffer (3 mM KCl; 1.5 mM KH$_2$PO$_4$; 68 mM NaCl; 9 mM NaH$_2$PO$_4$) to remove excess blood and cellular debris. The cell pellet was washed three times in low-salt washing buffer and resuspended in boiling SDS-lysis buffer (10% SDS, 0.15 M Tris-HCl, 1% β-mercaptoethanol) with 10% DNase/RNase solution, and vortexed for 30 sec. The lysates were then boiled for 30 sec and immersed in liquid nitrogen (72). Samples were lyophilized on a Speedvac drier and resuspended in rehydration buffer (8M urea, 2% DTT, 2% CHAPS). The lysate was cleared of cell debris by centrifugation at 13,000 rpm for 10 min at room temperature. The supernatant was then transferred to a new tube. To the total protein sample, an equal volume of ammonium sulfate was added and mixed for 30–60 min at 4°C. The sample was centrifuged at 3,000 × g for 15 min at 4°C. The supernatant was removed and discarded, with the pellet washed five times in low-salt washing buffer. The protein pellet was resuspended in rehydration buffer (8 M urea, 2% DTT, 2% CHAPS) and quantified.

Transactivation Studies

COS-1 and P19 cells, both with low endogenous VDR, were maintained in DMEM with 10% FCS. For comparison of transactivation by VDRA and VDRB1 cells were seeded in 24-well plates at a density of 2 × 10$^4$ cells per well for cytomegalovirus (CMV)-VDR, or 10$^5$ cells per well for GFP-VDR studies, in DMEM with 2% charcoal-stripped FBS. Cells were transfected 24 h later in serum-free medium using FUGENE6 with 540 ng DNA/well. Transfected DNA consisted of 20 ng pRC/CMV-VDR isoform expression plasmid, 250 ng of rat 24-hydroxylase promoter-luciferase reporter construct (24(OH)ase-Luc), 230 ng empty pRC/CMV vector, and 40 ng pRous sarcoma virus-β-galactosidase reporter construct. After 5 h cells were treated with 1 or 10 nM 1,25-(OH)$_2$D$_3$ or vehicle (isopropanol), as indicated, in DMEM with 2% charcoal-stripped FBS, incubated at 37°C for 18 h, and then lysed in cell lysis buffer. Reporter activity was determined by the Luciferase Reporter System assay (Promega Corp.). Results are expressed as mean ± SEM from at least three separate
transfections, each in triplicate, and normalized to β-galactosidase level (Tropix, PE Applied Biosystems, Bedford, MA).

Fluorescence Microscopy and Immunocytochemistry

For fluorescence microscopy, $10^5$ COS-1 cells per well in two-well Labtek-II chamberwell slides (NUNC, Naperville, IL) were transfected with 2 μg of pVDRA-EGFP or pVDRB1-EGFP or pEGFP. After 18 h, cells were fixed with 2% paraformaldehyde/PBS for 30 min, washed three times with PBS, and coverslipped. For each construct, 180 cells were examined. Colocalization studies (74) used mouse monoclonal antibodies H5 against hyperphosphorylated RNA PolIIo (28) (Babco, Richmond, CA), the rabbit anti-transcription factor IID (TBP) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), the spliceosome assembly factor SC-35 (Sigma, St. Louis, MO), PML nuclear bodies (5E10- GeneTex, ), or p80 Coilin rabbit antisera (kind gift from Prof. A. Lamond) as primary antibody and Alexa Fluor 594 antimouse, and 594 antirabbit secondary antibodies (Molecular Probes, Inc., Eugene, OR). Nuclear integrity was confirmed for all dual-labeling experiments by 4,6-diamidino-2-phenylindole staining (Roche Molecular Biochemicals). Cells were photographed on a DMR microscope (Leica Corp. Microsystems, Nussloch, Germany) using standard fluorescein isothiocyanate/Texas Red filter sets and 100× objective with oil immersion.

Acknowledgments

We thank Prof. Angus Lamond for the p80 coilin antiserum, Drs. G. Corthals and V. Wasinger for their assistance with the protein extractions, and J. Flanagan and other members of the Bone and Mineral Program for helpful discussions.

Received December 12, 2000. Accepted May 24, 2001. Address requests for reprints to: Dr. Edith Gardiner, Bone and Mineral Research Program, Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, Sydney, NSW 2010, Australia, E-mail: e.gardiner@garvan.unsw.edu.au.

This work was supported by the National Health and Medical Research Council of Australia, and an Australian Postgraduate Award (APA).

Current Address: Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

References


7. Nappali S, Friant S, Nakshatri H, Chambon P 1993 RARs and RXRs: evidence for two autonomous transactivation functions (AF-1 and AF-2) and heterodimerization in vivo. EMBO J 12:2349–2360


9. Sartorius CA, Tung L, Takimoto GS, Horwitz KB 1993 Antagonist-occupied human progesterone receptors bound to DNA are functionally switched to transcriptional agonists by cAMP. J Biol Chem 268:9262–9296

10. Sartorius CA, Melville MY, Howland AR, Tung L, Takimoto GS, Horwitz KB 1994 A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isofrom. Mol Endocrinol 8:1347–1360


31. Gall JG, Bellini M, Wu Z, Murphy C 1999 Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. Mol Biol Cell 10:4385–4402


39. Gall JG, Bellini M, Wu Z, Murphy C 1999 Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. Mol Biol Cell 10:4385–4402


47. Hadzic E, Desai-Yajnik V, Helmer E, et al. 1995 A 10-amino-acid sequence in the N-terminal A/B domain of thyroid hormone receptor α is essential for transcriptional activation and interaction with the general transcription factor TFIIB. Mol Cell Biol 15:4507–4517

48. Ford J, McEwan IJ, Wright AP, Gustafsson JA 1997 Involvement of the transcription factor IID protein complex in gene activation by the N-terminal transactivation domain of the glucocorticoid receptor in vitro. Mol Endocrinol 11:1467–1475
42. Banaihmrad A, Ha I, Reinberg D, Tsai S, Tsai MJ, O'Malley BW 1993 Interaction of human thyroid hormone receptor β with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone. Proc Natl Acad Sci USA 90:8832–8836


44. Glimeur C, Bailly M, Gyhsdael J 1989 The c-erbA α-encoded thyroid hormone receptor is phosphorylated in its amino terminal domain by casein kinase II. Oncogene 4:1247–1254

45. Stoltz C, Vachon MH, Trottier E, Dubois S, Paquet Y, H9251


68. Baumann CT, Ma H, Wolford R, et al. 2001 The glucocorticoid receptor interacting protein 1 (GRIP1) localizes in discrete nuclear foci that associate with ND10 bodies and are enriched in components of the 26S proteasome. Mol Endocrinol 15:485–500


